
PROFESSOR YONGZHE LI (Orcid ID : 0000-0002-8267-0985)

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Brief Report: Anti-phospholipid antibodies in critically ill patients with Coronavirus Disease 2019 (COVID-19)

Meng Xiao^{1*}, M.S., Yan Zhang^{2*}, M.D., Shulan Zhang^{3*}, M.D., Xuzhen Qin^{1*}, M.D., Peng Xia⁴, M.D., Wei Cao⁵, M.D., Wei Jiang⁶, M.D., Huan Chen⁷, M.D., Xin Ding⁷, M.D., Hua Zhao⁷, M.D., Hongmin Zhang⁷, M.D., Chunyao Wang⁶, M.D., Jing Zhao⁸, M.D., Xuefeng Sun⁸, M.D., Ran Tian⁹, M.D., Wei Wu⁹, M.D., Dong Wu¹⁰, M.D., Jie Ma⁴, M.D., Yu Chen¹, B.S., Dong Zhang¹, M.S., Jing Xie⁵, Ph.D., Xiaowei Yan⁹, M.D., Xiang Zhou⁷, M.D., Zhengyin Liu⁵, M.D., Jinglan Wang⁸, M.D., Bin Du⁶, M.D., Yan Qin⁴, M.D., Peng Gao⁹, M.D., Minya Lu¹, M.D., Xin Hou¹, Ph.D., Xian Wu¹, Ph.D., Huadong Zhu¹¹, M.D., Yingchun Xu¹, M.D., Wen Zhang³, M.D., Taisheng Li⁵, M.D., Fengchun Zhang³, M.D., Yongqiang Zhao², M.D., Yongzhe Li^{1#}, M.D., Shuyang Zhang^{9#}, M.D.

*These authors contributed equally to the study.

#These authors contributed equally as senior authors.

¹Department of Laboratory Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, 100730

²Department of Hematology, Peking Union Medical College Hospital, Chinese Academy of Medical

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Sciences, Beijing, 100730

³Department of Rheumatology and Clinical Immunology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, 100730

⁴Department of Nephrology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, 100730

⁵Department of Infectious Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, 100730

⁶Department of Medical ICU, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, 100730

⁷Department of Intensive care Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, 100730

⁸Department of Pulmonary and Critical Care, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, 100730

⁹Department of Cardiology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, 100730

¹⁰Department of Gastroenterology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, 100730

¹¹Department of Emergency Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, 100730

Correspondence: Shuyang Zhang, Department of Cardiology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, 1# Shuai Fu Yuan, Beijing 100730 China. Phone: +86-10-69156810. E-mail: shuyangzhang103@163.com.

or Yongzhe Li, Department of Laboratory Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, 1# Shuai Fu Yuan, Beijing 100730 China. Phone: +86-10-69159713.

E-mail: yongzhelipumch@126.com

Subtitle: aPLs in Critically Ill Patients With COVID-19

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Abstract

Objectives: Coagulopathy is one of the characteristics of critically ill patients with Coronavirus Disease 2019 (COVID-19). Antiphospholipid antibodies (aPLs) contribute to coagulopathy, but their role in COVID-19 remains unclear. We aimed to determine the prevalence and characteristics of aPLs in patients with COVID-19.

Methods: Sera collected from 66 critically ill and 13 non-critically ill patients with COVID-19 were tested for anti-cardiolipin (aCL) and anti- β 2-glycoprotein 1 (a β 2GP1) (IgG, IgM, and IgA) and IgG a β 2GP1-D1 by the chemiluminescence assay (CIA) and IgM and IgG anti-phosphatidylserine/prothrombin (aPS/PT) by ELISA.

Results: aPLs were detected in 47.0% of critically ill patients (31/66), but not in patients with non-critical conditions. IgA a β 2GP1 was the most common aPL, present in 28.8% (19/66) critically ill patients, followed by IgA aCL (25.8%, 17/66) and IgG a β 2GP1 (18.2%, 12/66). For multiple aPLs, IgA a β 2GP1+IgA aCL was the most common type (22.7%, 15/66), followed by IgA a β 2GP1+IgA aCL+IgG a β 2GP1 (15.2%, 10/66). aPLs emerge around 35-39 days post-disease onset. Dynamic analysis of aPLs revealed 4 patterns based on persistence or transient appearance of the aPLs. Patients with multiple aPLs displayed significantly higher incidence of cerebral infarction ($p=0.023$).

Conclusions: aPLs were common in critically ill patients. Multiple medium or high levels aPLs may help identify patients at risk of developing cerebral infarction. aPLs may be transient and disappear within a few weeks, but in genetically predisposed patients, COVID-19 may trigger the development of "COVID-19-induced-APS-like-syndrome". Long-term follow-up on COVID-19 patients positive for aPLs would be of great importance.

Keywords: COVID-19, antiphospholipid antibodies, coagulopathy, critically ill patients,

We and others have observed that critically ill patients with Coronavirus Disease 2019 (COVID-19) are associated with a proinflammatory and hypercoagulable state and increased risk of thrombotic events (i.e., pulmonary embolism and cerebral infarction), which are characterized by marked elevations of D-dimers(1-6). Currently, the etiology leading to hypercoagulability in COVID-19 remains unclear. Antiphospholipid syndrome (APS), an autoimmune disorder characterized by the presence of antiphospholipid antibodies (aPLs) and a wide series of clinical manifestations, from recurrent arterial and/or venous thrombotic events to recurrent fetal loss. aPLs have long been considered as one of the contributors to the hypercoagulable states and following thrombotic events. In addition to the pathogenic role in APS, aPLs are crucial to the diagnosis of APS. The 2006 criteria recommend lupus anticoagulant (LA), IgM and/or IgG anti-cardiolipin (aCL) and IgM and/or IgG anti- β 2-glycoprotein 1 (a β 2GP1) antibodies for routine tests. In addition, 14th International Congress on Antiphospholipid Antibodies Technical Task Force Report highlighted non-criteria aPLs, including IgA aCL, IgA a β 2GP1, IgM/IgG anti-phosphatidylserine/prothrombin (aPS/PT) and a β 2GP1 domain 1 (D1), are associated to the APS, especially seronegative APS (SNAPS) (7, 8). We have previously reported the presence of aPLs in 3 critically ill patients with COVID-19 (9). However, it remains unclear whether these aPLs are pathogenic or whether they are persistent. In this study, we summarize the prevalence and characteristics of aPLs in 66 critically ill patients with COVID-19 and provide clinical features based on the presence of aPLs.

Materials

Settings and Patients

Consecutive critically ill patients with suspected COVID-19, who were admitted to an intensive care unit (ICU) designated for patients with COVID-19, were included in this cross-sectional study. This unit, which was managed by a multidisciplinary team from Peking Union Medical College Hospital

(PUMCH) in the Sino-French New City Branch of Tongji Hospital in Wuhan, China, was set up on an emergency basis to accept the most critically ill patients during the outbreak of COVID-19. Patients inclusion criteria were all the patients managed in our ICU. Patients exclusion criteria were: 1). non-COVID-19 patients; 2). COVID-19 patients who did not test for aPLs. A total of 66 critically ill patients with COVID-19 were finally included in this study. COVID-19 patients visiting the fever clinic in PUMCH in Beijing, China, were also included. The inclusion criteria for those patients were all the consecutive patients who visited our fever clinic in PUMCH in Beijing. Exclusion criteria were non-COVID-19 patients. A total of 13 patients with COVID-19 were finally included. All these 13 patients were non-critically ill patients. The diagnosis of SARS-CoV-2 infection was confirmed in all the patients by reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assay or serologic testing according to the Chinese Recommendations for Diagnosis and Treatment of Novel Coronavirus Infection (Pilot 7th version). The clinical characteristics and laboratories parameters at time of admission, which were collected from electronic medical records, are listed in Table 1. The study was approved by the Research Ethics Commission of PUMCH and the requirement for informed consent was waived by the Ethics Commission (ZS-2303).

Serum aPLs Determination

Serum aCL and a β 2GP1 (IgG, IgM, and IgA) and IgG a β 2GP1-D1 were determined by the chemiluminescence assay (CIA) (INOVA)(10). The cutoff values for positivity were set >20 U based on manufacturer's recommendations. IgG/IgM aPS/PT were determined by ELISA (INOVA)(11). The cutoff values for positivity were set >30 U based on manufacturer's recommendations.

Lupus anticoagulant (LA)

The detection of LA in human citrated plasma was performed by the HemosIL dRVVT Screen and HemosIL dRVVT Confirm assays, as recommended by the International Society on Thrombosis and Haemostasis (ISTH).

Statistical analysis

Mann-Whitney U test, χ^2 test, or Fisher's exact test were utilized to compare differences between aPL positive group and aPL negative group where appropriate. A Kruskal-Wallis test followed by Dunnett's T2 test was used to compare aPLs negative group, Single/multiple^{lo} group and Multiple^{med/hi} group. A two-sided α of less than 0.05 was considered statistically significant. Statistical analyses were performed on SPSS 20.0 package (SPSS Inc.).

Results

We first determined the prevalence and characteristics of aPLs in patients with COVID-19. When the manufacturer's recommended cut-off value of >20 CU was utilized, aPLs were detected in 47.0% of critically ill patients (31/66), but not in patients with non-critical conditions (Table 2). Previous study has shown that moderate to high titers of aPLs display better clinical significance (12). As such, we re-analyzed the prevalence of aPLs utilizing the cut-off value of >40 CU. aPLs were present in 31.8% of critically ill patients (21/66). We next assessed the prevalence of each aPL. For single aPL, IgA α 2GP1 was the most common type of aPLs, present in 28.8% (19/66) of critically ill patients and 61.3% (19/31) of aPLs-positive patients, respectively, followed by IgA aCL (25.8% (17/66) and 54.8% (17/31), respectively), and IgG α 2GP1 (18.2% (12/66) and 38.7% (12/31), respectively). For multiple aPLs, IgA α 2GP1+IgA aCL was the most common type (22.7%, 15/66), followed by IgA α 2GP1+IgA aCL+ IgG α 2GP1 (15.2%, 10/66) and IgA aCL+IgG aCL+IgA α 2GP1+IgG α 2GP1 (6.1%, 4/66). Lupus anticoagulant was tested in all the 66 critically-ill patients, among whom, LA was positive in 2 patients. These findings suggested that COVID-19 preferentially induced IgA isotype aPLs and to a less extent, the IgG isotype aPLs.

Next, we determined when the aPLs emerged in those patients. Among the 31 aPL-positive patients, we had sera from 10 patients who were negative for aPLs at early time-point and positive for aPLs at

later time-point. Analysis of those patients revealed that aPLs emerged around a median time of 39 days post-disease onset (Supplemental table 1). Taken together, these findings show that aPLs emerge at a later time-point, suggesting critically ill patients with longer disease duration are likely to have aPLs.

Dynamic changes in the levels of aPLs during COVID-19 in critically ill patients were further investigated. Due to the retrospective nature, multiple time-points of aPLs results were only obtained from 6 patients (Figure 1). Generally, the levels and types of aPLs increased from a single type with low titers to multiple types with high titers. For the later time-points, those 6 patients exhibited different patterns. In patient 1, medium levels of IgG a β 2GP1 were maintained despite interventions with plasma exchanges (Figure 1A). In patients 2 and 3, medium levels of IgA a β 2GP1+IgA aCL were maintained after a transient appearance of IgG a β 2GP1 (Figure 1B). In patients 4 and 5, the aPLs were transient and disappeared at later time-points (Figure 1C). In patient 6, high levels of IgA aCL+IgA a β 2GP1+IgG a β 2GP1 were maintained for around two weeks (Figure 1D). These results suggest that the levels of aPLs are fluctuated and exhibit different dynamic patterns among different patients during COVID-19.

Last, we assessed the clinical relevance of aPLs in critically ill patients with COVID-19 (Table 1). As mounting evidence suggest that multiple aPL positivity or moderate to high titers of aPLs display better clinical values compared to single aPL positivity or low titers of aPLs. We divided aPLs positive group into single/multiple^{lo} group (patients positive for single aPL or positive for more than one aPLs but the values of all aPLs was at low titers (≤ 40 CU), as previously described (13)) and multiple^{med/hi} group (patients positive for more than one aPLs and the value of at least one aPL was at moderate titers (>40 CU)). The three critically ill groups displayed similar clinical and laboratory features, but the multiple^{med/hi} group showed significantly higher incidence of cerebral infarction compared to

aPL-negative group (0 vs 33.3%, $p=0.023$), suggesting that aPLs may be helpful in predicting the occurrence of cerebral infarction during COVID-19.

Discussion

The full spectrum of COVID-19 is still under intense investigation, but increasing evidence suggest that most critically ill patients suffer from coagulopathy (1-3). aPLs have been considered as one of the mechanisms leading to a proinflammatory and hypercoagulable state. In this study, we found that aPLs were present in a substantial number of critically ill patients. Although it remains unclear whether aPLs contribute to the hypercoagulable state in COVID-19, our findings suggest a possibility that aPLs may be implicated in this process.

Infection-induced aPL production has been widely acknowledged (14, 15). Of particular interest is that we found IgA, an isotype specialized in mucosal immunity, was the most common aPLs isotype. As COVID-19 mainly affects pulmonary and intestinal mucosa, the preferential production of IgA isotype may be associated with the breakage of mucosal immune tolerance. IgA a β 2GPI preferentially target the C-terminal portion of β 2GPI (domain 4 and 5) (16), thus the presence of IgA aPLs may suggest a novel subgroup of clinically relevant APS in critically ill patients with COVID-19. Interestingly, we found that although IgA aCL and IgA a β 2GPI transiently appeared in a subgroup patient, they persisted in another subgroup patients. Unfortunately, we could not perform long-term follow-up. A prospective evaluation of those aPLs in COVID-19 patients is in great need to investigate whether these are persistent and pathogenic or require long-term anticoagulants.

While it remains unclear whether IgA aPLs are pathogenic in APS, *in vivo* mouse studies demonstrated that IgA a β 2GPI induced significantly larger thrombi and higher tissue factor (TF) levels

compared to controls (17). IgA a β 2GPI are significantly and independently associated with arterial thrombosis and all thrombosis in patients with systemic lupus erythematosus (SLE) and APS (17). In addition, IgA a β 2GPI has been described as an independent risk factor for acute myocardial infarction (16, 18) and acute cerebral ischaemia (19). In this study, we found that patients with multiple aPLs, including IgA and IgG a β 2GPI and IgA and IgG aCL, displayed significantly higher incidence of cerebral infarction. Unfortunately, due to the critical condition of those patients as well as the limitation of the isolation ward, a large number of patients could not be screened by ultrasound, thus many thrombotic events may be underrepresented. It is also worth mentioning that the patients who developed cerebral infarction may have already had atherosclerosis and aPLs. It would be of great interest to assess whether multiple medium or high levels aPLs may help identify patients at risk of developing cerebral infarction in critically ill patients with COVID-19 in future studies.

This study has several limitations. In the analysis of the time-point for the emergence of aPLs, due to the retrospective nature of this study, for some patients, we only had one time-point, and for other patients, although we had more than one time-points, aPLs were already positive in the early time-point. Thus, we ended up with only 10 patients for the following analysis. The small sample size may bring analytical bias. Further prospective studies on the time-point of the emergence of aPLs are needed.

In conclusion, clinical significance of aPLs in critically ill patients with COVID-19 remains to be determined. In some patients, transient rises in aPL may be accompanied by thrombotic complications (15). It is worthy to note that, although in some patients, these antibodies may be transient and disappear within a few weeks, in other genetically predisposed patients, COVID-19 may trigger the development of “COVID-19-induced-APS-like-syndrome”. Long-term follow-up on COVID-19 patients positive for aPLs would be of great importance.

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Conflicts of Interest

The authors declare no competing financial interests.

Figures

Figure 1. Dynamic changes in the levels of aPLs during COVID-19 infection in critically ill patients (A). Patient 1, medium levels of IgG a β 2GP1 persisted after a transient appearance of IgA a β 2GP1+IgA aCL. (B). Patient 2 (left panel) and patient 3 (right panel), medium levels of IgA a β 2GP1+IgA aCL persisted after a transient appearance of IgG a β 2GP1. (C). Patient 4 (left panel) and patient 5 (right panel), transient appearance of aPLs. (D). Patient 6, high levels of IgA aCL+IgA a β 2GP1+IgG a β 2GP1 persisted.

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Table 1. Demographic, clinical characteristics and laboratory findings of patients infected with COVID-19

Characteristics	Critically-ill (n=66)			p-value	Non-critically-ill (n=13)
	aPLs negative (n=35)	aPLs positive (n=31)			
		Single/multiple ^{lo} (n=16)	Multiple ^{med/hi} (n=15)		
Age, years	64.5±12.3	66.5±13.3	65.2±7.5	0.850	35.2±19.3
Gender, female/male	17/18	5/11	5/10	0.402	7/6
Comorbidity					
Hypertension, n (%)	17 (48.6)	8 (50.0)	8 (53.3)	0.953	0
Diabetes, n (%)	6 (17.1)	3 (18.8)	4 (26.7)	0.657	0
Coronary heart disease, n (%)	8 (22.9)	0	2 (13.3)	0.092	0
Lung disease, n (%)	5 (14.3)	1 (6.2)	1 (6.7)	0.660	0
Carcinoma, n (%)	1 (2.9)	1 (6.2)	2 (13.3)	0.247	0
Chronic kidney disease, n (%)	0	0	1 (6.7)	N/A	0
Chronic liver disease, n (%)	4 (11.4)	0	1 (6.7)	0.499	1 (9.1)
Autoimmune diseases, n (%)	2 (5.7)	0	0	1.000	0
Thrombotic history					
Cerebral infarction, n (%)	4 (11.4)	3 (18.8)	2 (13.3)	0.889	0
Myocardial infarction, n (%)	1 (2.9)	1 (6.2)	0	0.723	0
Other thrombotic events	0	0	0	N/A	0
Symptoms on admission					
Fever (temperature ≥37·3°C), n (%)	31 (88.6)	14 (87.5)	13 (86.7)	1.000	8 (61.5)
Cough, n (%)	32 (91.4)	12 (75.0)	10 (66.7)	0.079	9 (69.2)
Sputum, n (%)	12 (34.3)	6 (37.5)	4 (26.7)	0.885	0
Dyspnea, n (%)	28 (80.0)	15 (93.8)	11 (73.3)	0.336	0
Myalgia, n (%)	9 (25.7)	4 (25.0)	3 (20.0)	1.000	0
Fatigue, n (%)	15 (42.9)	3 (18.8)	8 (53.3)	0.116	0
Diarrhoea, n (%)	12 (34.3)	3 (18.8)	2 (13.3)	0.271	0
Headache, n (%)	6 (17.1)	2 (12.9)	3 (20.0)	0.911	0
Nausea or vomiting, n (%)	9 (25.7)	1 (6.2)	3 (20.0)	0.276	0
Disease severity status					
General, n (%)	0	0	0		12 (92.3)
Severe, n (%)	0	0	0		1 (7.7)
Critical, n (%)	35 (100)	16 (100)	15 (100)		0
ARDS, n (%)	12 (34.3)	7 (43.8)	6 (40.0)	0.794	0
Respiratory failure, n (%)	23 (65.7)	13 (81.2)	11 (73.3)	0.516	0
Laboratory findings on admission					
White blood cell count, 10 ⁹ /L	13.5±6.3	14.3±7.1	13.5±7.2	0.921	7.3±2.4
Total neutrophil count, 10 ⁹ /L	12.1±5.9	12.1±6.8	12.1±6.7	0.999	3.3±1.6

Total lymphocyte count, 10 ⁹ /L	0.6±0.6	0.8±0.6	0.7±0.4	0.599	1.7±0.7
Red blood cell count, 10 ¹² /L	3.5±0.9	4.1±1.2	3.5±0.6	0.130	4.5±0.6
Platelets, 10 ⁹ /L	150.6±102.9	177.9±83.9	185.0±83.2	0.419	223.0±62.8
Hemoglobin, g/L	108.5±23.7	120.8±28.3	107.3±22.2	0.211	134.1±14.8
ALT, U/L	38.1±64.2	68.4±165.7	28.9±19.5	0.456	14.2±7.5
AST, U/L	37.8±30.7	180.4±583.7	34.0±18.7	0.224	N.D.
LDH, U/L	510.1±292.9	533.6±458.8	447.9±218.5	0.750	N.D.
Creatinine µmol/L	106.6±125.9	74.6±40.5	76.3±37.2	0.427	52.3±23.5
eGFR (ml/min/1.73m ²)	83.0±35.0	89.8±31.7	86.9±24.8	0.770	N.D.
High-sensitive cardiac troponin I, pg/mL	594.9±2410.0	607.0±1921.2	215.7±497.6	0.812	N.D.
NT-proBNP, pg/ml	3029.6±5306.6	1756.2±2189.2	2016.9±2217.6	0.536	N.D.
Prothrombin time, s	17.6±3.5	17.6±7.5	16.1±1.0	0.561	N.D.
APTT, s	45.4±21.0	45.8±7.6	41.36±6.44	0.677	N.D.
Fibrinogen, g/L	3.6±2.1	4.8±1.6	4.5±1.2	0.064	N.D.
D-dimer, µg/L	10.9±8.8	10.2±9.0	8.9±7.6	0.744	N.D.
Procalcitonin, ng/mL	0.8±1.9	0.3±0.4	1.2±2.0	0.335	0.2±0.1
hsCRP, mg/L	88.7±84.3	98.1±57.6	99.5±51.8	0.855	N.D.
IL-6, pg/ml	289.5±877.5	277.3±539.1	103.1±125.3	0.671	N.D.
Treatments					
Corticosteroids, n (%)	27	12	10 (66.7)	0.703	N/A
Intravenous immunoglobulin, n (%)	18	8	11 (73.3)	0.325	N/A
Non-invasive mechanical ventilation, n (%)	17	11	7 (46.7)	0.348	N/A
Invasive mechanical ventilation, n (%)	28	14	15 (100)	0.193	N/A
Anticoagulant therapy, n (%)	19	12	9 (60.0)	0.372	N/A
ECMO, n (%)	3	1	3 (15.0)	0.490	N/A
Thrombotic events during COVID-19 infection					
Arterial thrombosis					
Cerebral infarction, n (%)	0	0	5 (33.3)	0.010	0
Myocardial infarction, n (%)	0	0	1 (6.7)	0.227	0
Venous thrombosis					
Large vein, n (%)	0	0	2 (13.3)	0.227	0
Distal Vein, n (%)	10	3	4 (26.7)	0.870	0

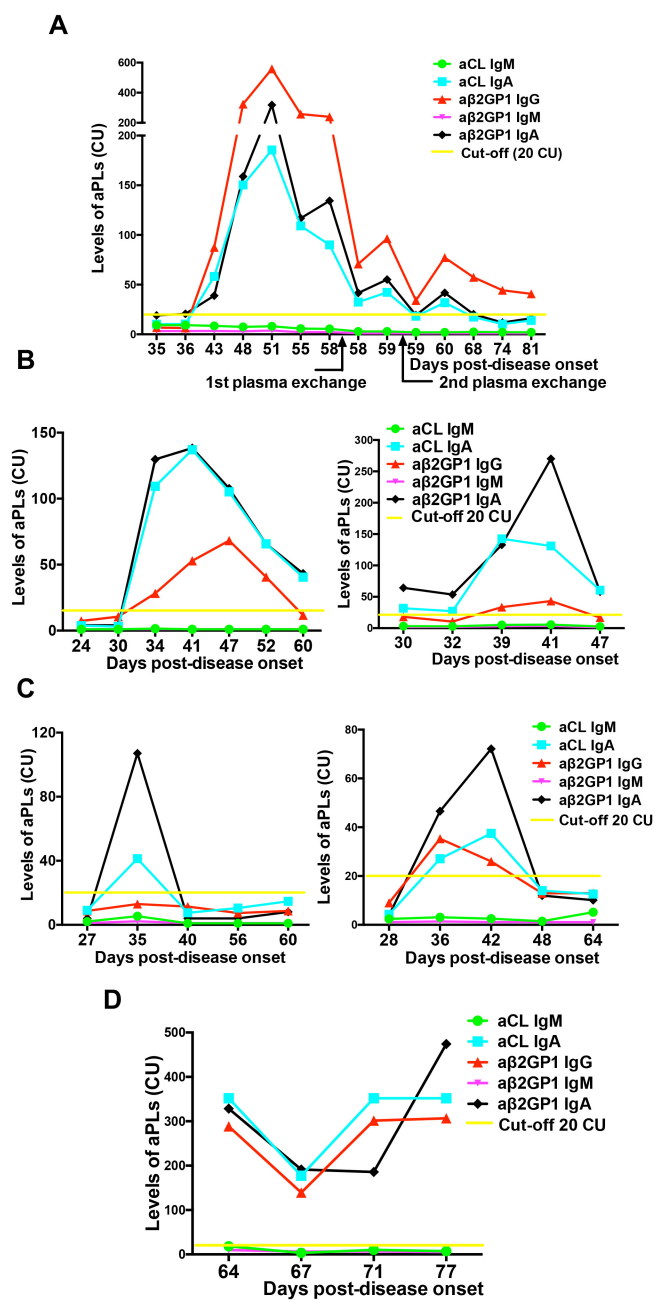
ALT, alanine transaminase; aPLs, Antiphospholipid antibodies; APTT, activated partial thromboplastin time; ARDS, acute respiratory distress syndrome; AST, aspartate transaminase; CHD, coronary heart disease; eGFR, estimated glomerular filtration rate; HFNC, high-flow nasal cannula; hsCRP, high sensitivity C-reactive protein; IMV, invasive

mechanical ventilation; IVIG, intravenous immunoglobulin; LDH, lactate dehydrogenase; NIMV, non-invasive mechanical ventilation; N/D, not determined; N/A not applicable; NT-proBNP, N terminal pro B type natriuretic peptide. Single/multiple^{lo} refers to patients positive for a single aPL or positive for more than one aPLs but the values of all aPLs was ≤ 40 CU. Multiple^{med/hi} refers to patients positive for more than one aPLs and the value of at least one aPL was >40 CU. *p* value was calculated with a Kruskal-Wallis test followed by Dunnett's T2 test. For cerebral infarction during COVID-19 infection, *p*-value between aPLs negative group and multiple aPLs positive group was 0.023, *p*-value between aPLs single positive group and multiple aPLs positive group was 0.101.

Table 2. Prevalence and characteristics of aPLs in patients with COVID-19

Antiphospholipid antibodies (aPLs)	Critically-ill (n=66)	Non-Critically- ill (n=13)
Overall prevalence in any aPLs, n (%)	31 (47.0)	0
IgA aCL, n (%)	17 (25.8)	0
IgG aCL, n (%)	4 (6.0)	0
IgM aCL, n (%)	2 (3.0)	0
Lupus anticoagulant, n (%)	2 (3.0)	0
IgA a β 2GP1, n (%)	19 (28.8)	0
IgG a β 2GP1, n (%)	12 (18.2)	0
IgM a β 2GP1, n (%)	1 (1.5)	0
IgG a β 2GP1-D1, n (%)	2 (3.0)	0
IgM aPS/PT, n (%)	7 (10.6)	0
IgG aPS/PT, n (%)	0	0
IgA aCL+IgA a β 2GP1, n (%)	15 (22.7)	0
IgM aCL+IgM a β 2GP1, n (%)	1 (1.5)	0
IgA a β 2GP1+IgG a β 2GP1, n (%)	1 (1.5)	0
LA+IgA aCL+IgA a β 2GP1, n (%)	1 (1.5)	0
IgA aCL+IgA a β 2GP1+IgG a β 2GP1, n (%)	10 (15.2)	0
IgA aCL+IgG a β 2GP1+IgM aCL, n (%)	1 (1.5)	0
IgA aCL+IgA a β 2GP1+IgM aPS/PT, n (%)	1 (1.5)	0
IgA aCL+IgG aCL+IgA a β 2GP1+ IgG a β 2GP1, n (%)	4 (6.1)	0
LA+IgA aCL+IgG aCL+IgA a β 2GP1+ IgG a β 2GP1, n (%)	1 (1.5)	0

The cutoff values for positivity in all aPLs except IgM/IgG aPT/PS were set >20 U based on the recommendations of the manufacturer. The cutoff values for positivity in IgM/IgG aPT/PS were set >30 U based on the recommendations by the manufacturer. a β 2GP1, anti- β 2-glycoprotein 1 antibodies; a β 2GP1-D1, anti- β 2-glycoprotein domain 1 antibodies; aCL, anticardiolipin antibodies; aPS/PT, anti-phosphatidylserine/prothrombin, LA, lupus anticoagulants; N/D, not determined



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